Topical Treatment with a New Matrix Therapy Agent (RGTA) for the Treatment of Corneal Neurotrophic Ulcers

Abdelouahab Aifa, Julie Gueudry, Alexandre Portmann, Agnès Delcampe, and Marc Muraine

Purpose. Neurotrophic keratopathy is a degenerative disease of the corneal epithelium resulting from impaired corneal innervation, possibly leading to perforation. We aimed to assess the efficacy and tolerance of a new matrix therapy agent (RGTA, Cacicol20), mimicking heparan sulfates, for the management of neurotrophic keratopathy.

Methods. We carried out an uncontrolled, prospective, single-center clinical study on 11 patients (11 eyes) with severe corneal neurotrophic ulcers, despite the use of preservative-free artificial tears, for 15 days. Patients were treated with RGTA eye drops, instilled at a dosage of one drop in the morning, on alternate days. Evolution and follow-up during treatment were evaluated by slit-lamp examination, photography, fluorescein-dye testing, tests of corneal sensitivity, and best corrected visual acuity. The main outcome measures for each patient were healing of the corneal surface and best corrected visual acuity before and after RGTA therapy.

Results. Eight patients displayed complete corneal healing after a mean period of 8.7 weeks (range; 1 to 22 weeks). Mean ulcer area decreased significantly, from 11.12% to 6.37% ($P = 0.048$) in the first week, and to 1.56% ($P = 0.005$) at 1 month. Treatment failure was observed in three cases, requiring amniotic membrane transplantation in two patients and penetrating keratoplasty in one patient. At the end of the study, none of the patients displayed significant improvement in visual acuity. There were no systemic or local side effects of treatment.

Conclusions. RGTA seems to be a potentially useful, alternative, noninvasive therapeutic approach in neurotrophic keratopathy management. However, randomized studies are necessary. (*Invest Ophthalmol Vis Sci.* 2012;53:8181–8185) DOI: 10.1167/iovs.12-10476

The integrity of the corneal epithelium and the process of reepithelialization are dependent on innervation of cornea. Underlying mechanisms have not been fully elucidated, but this integrity seems to be linked to trophic factors, including growth factors, such as nerve growth factor (NGF).[1] Loss of sensitivity leads to a decrease in the number of limbic stem cells, as well as slowing of metabolism and mitosis. As a consequence, corneal healing is slowed or may not occur. Corneal anesthesia may lead to neurotrophic keratopathy, such as superficial punctate keratitis and corneal ulcer, which can result in corneal opacification, irregular astigmatism due to corneal scarring, or even corneal perforation. Hypoesthesia and anesthesia may be due to viral infection, chemical burns, surgical or laser treatment, corneal dystrophy, diabetes, or trigeminal nerve damage. Corneal neurotrophic ulcers remain difficult to treat.[2] Treatment consists of instillation of artificial tears and elimination of toxic agents, particularly preservatives; however, such treatment may be insufficient. Other drug-based and surgical treatments have been described, such as autologous serum, conjunctival flaps, tarsorrhaphy, and amniotic membrane transplantation.[3] Other approaches are also being studied, including the application of growth factors, such as NGF, which is considered as promising for use in neurotrophic keratopathy treatment.[4]

Over the past few years, a new type of matrix therapy agent (ReGeneraTing Agent [RGTA]) has provided encouraging results, accelerating the healing of chronic skin ulcers of diabetic or vascular origin.[5,6] Large polymers replace destroyed heparan-sulfate molecules, thereby creating a cellular microenvironment favorable to healing. In the domain of ophthalmology, RGTA has been reported to show encouraging results in the treatment of corneal ulcers and dystrophies of various etiologies.[7] Furthermore, it was described in a case report concerning one patient with a neurotrophic ulcer.[8]

The aim of this study was to evaluate the efficacy and safety of RGTA eye drops in the treatment of resistant corneal neurotrophic ulcers. To our knowledge, we report here the first series of this type.

Patients and Methods

Study Design

We carried out an uncontrolled, prospective, single-center clinical study at Rouen University Hospital-Charles Nicolle (Rouen, France) between May and December 2011. We assessed the efficacy and safety of RGTA treatment in patients presenting a neurotrophic ulcer resistant to usual treatment and associated with complete corneal anesthesia. This study was in compliance with local institutional review board (CPP- Nord Ouest I, Rouen, France) requirements. The research adhered to the tenets of the Declaration of Helsinki.

Inclusion criteria were age older than 18 years, total corneal anesthesia, and neurotrophic ulcer failing to heal despite the exclusion of preservatives from treatment for at least 15 days. Treatments at the time of inclusion were preservative-free artificial tears, which had been administered to all patients for 15 days, and 2% cyclosporine eye drops, if appropriate. PCR was performed on the corneal scraping samples of all patients to test for the presence of herpes simplex virus 1 (HSV1), HSV2, and varicella zoster virus genomes and was to be negative.

Treatment

RGTA eye drops (Cacicol20, OTR^3, Paris, France), were instilled in the morning, as the first eye drop, on alternate days. Patients were

From the Ophthalmology Department, Rouen University Hospital-Charles Nicolle, Rouen, France.

Submitted for publication June 27, 2012; revised August 13 and September 30, 2012; accepted November 8, 2012.

Disclosure: **A. Aifa**, None; **J. Gueudry**, None; **A. Portmann**, None; **A. Delcampe**, None; **M. Muraine**, None

Corresponding author: Marc Muraine, Ophthalmology Department, Rouen University Hospital-Charles Nicolle, 1 rue de Germont, 76031 Rouen cedex, France; marc.muraine@chu-rouen.fr.

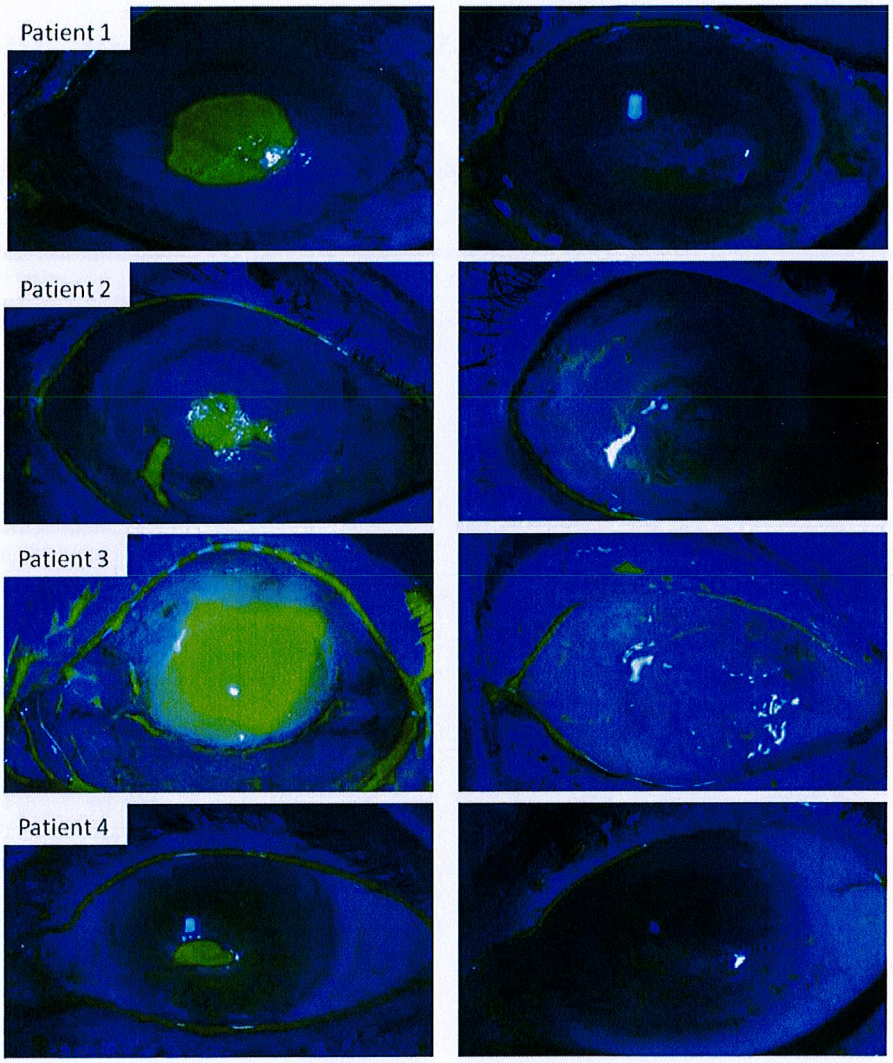

FIGURE 1. Corneal neurotrophic ulcers after RGTA treatment. *Left column:* before treatment; *right column:* after healing. Patient 1: bacterial keratitis, complete corneal healing after 15 days. Patient 2: chemical burn, complete corneal healing after 1 month. Patient 3: penetrating keratoplasty, complete corneal healing after 12 weeks. Patient 4: metaherpetic keratitis, complete corneal healing after 15 days.

otherwise treated with preservative-free artificial tears alone, and with 2% cyclosporine drops, administered twice daily, if appropriate (for the three patients who had undergone limbal allotransplantation). RGTA treatment was stopped as soon as complete healing was achieved.

Patient Follow-up

Patients attended follow-up consultations on days 7, 14, 21, and 28, and at months 2 and 3 after treatment initiation. Corrected visual acuity was measured at each follow-up visit. Slit lamp examinations were carried out, with photography of the anterior segment and fluorescein-dye testing. A corneal sensitivity test was performed with a small sponge at each follow-up visit.

Outcome Measures and Statistical Analysis

The main outcome measures for each patient were healing of the corneal surface and best corrected visual acuity before and after RGTA therapy. The area of the neurotrophic ulcer was determined, at each visit, by slit lamp photography, as proportion of the total area of cornea, with dedicated image analysis software (Image J, version 1.31, Wayne Rasband Research Service Branch, National Institute of Mental Health, Bethesda, MD). Visual acuity was transformed to the logarithm of the minimum angle of resolution acuity (LogMAR) for statistical analysis. Paired Student's *t*-tests were used to compare for each patient's best corrected visual acuity values before and after RGTA treatment and corneal ulcer area before and after RGTA treatment. Statistics were analyzed using GraphPad InStat software version 3.00 (GraphPad Software, San Diego, CA). A P value less than 0.05 was considered statistically significant.

RESULTS

Eleven eyes of 11 patients were included. Demographic characteristics are summarized in the Table. Complete corneal healing was observed in 8 (72.7%) of the 11 patients after 8.7 weeks (range: 1 to 22 weeks) (Fig. 1). Ulcer healing was centripetal, without de novo vascularization of the ulcer in four patients, but slight aggravation of preexisting vascularization was noted in the other four cases. The mean area of

Table. Demographic Characteristics of Patients with Corneal Neurotrophic Ulcer

	No. of Patients	
Patients	11	
Male/Female	3/8	
Mean age, y	58 (range, 22 to 83)	
Cause of ulcer		
Postinfectious keratitis	5	HSV1: 2 cases Bacterial keratitis: 2 cases Acanthamoeba keratitis: 1 case
Limbal allografts	3	After chemical or thermal burns
Emergency penetrating keratoplasty	1	After perforating bacterial keratitis
Neovascular glaucoma	1	
Complications of TEN	1	

TEN, toxic epidermal necrolysis.

neurotrophic ulcers, calculated as a proportion of the total area of the cornea, decreased significantly during follow-up (Fig. 2). The mean ulcer area decreased significantly, from 11.12% to 6.37% ($P = 0.0479$) in the first week, to 4.24% ($P = 0.0142$) at 2 weeks, 2.35% ($P = 0.0102$) at 3 weeks, and 1.56% ($P = 0.0054$) at 4 weeks. After 2 months, the mean area of corneal ulcer was only 1.41% of the total area of the cornea ($P = 0.0051$). After 3 months, it was only 0.33% of the total area of the cornea ($P = 0.0065$). Corneal ulcers healed after 1 week in one patient, after 2 weeks in two patients, between the fifth and sixth week in three patients, and after a longer period (12 weeks and 22 weeks) in two patients respectively (Fig. 3). Healing occurred in total absence of corneal sensitivity.

Three cases of treatment failure were recorded (Fig. 4). One patient presented a corneal perforation. Aggravation of neurotrophic ulcers was observed in two patients, the first one presenting corneal complications of acanthamoeba keratitis and the second presenting neovascular glaucoma. Both of these patients underwent amniotic membrane transplantation and one also underwent penetrating keratoplasty.

During follow-up, we observed only one case of recurrence after the total healing of a neurotrophic ulcer, 4 months after the end of treatment. Outcome was favorable after RGTA treatment reintroduction.

Mean corrected visual acuity did not differ significantly before and after treatment. Indeed, mean LogMAR visual acuity

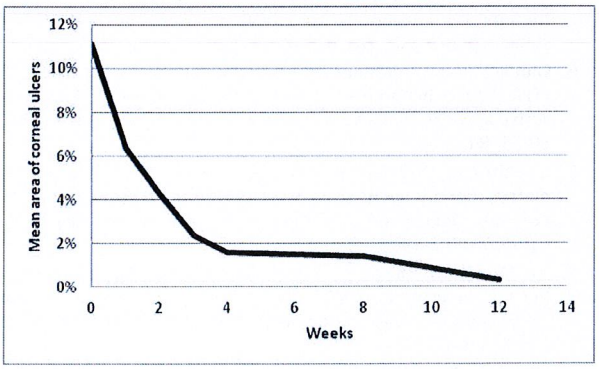

Figure 2. Area of the corneal neurotrophic ulcer (%) during RGTA treatment (weeks), in patients displaying complete corneal healing.

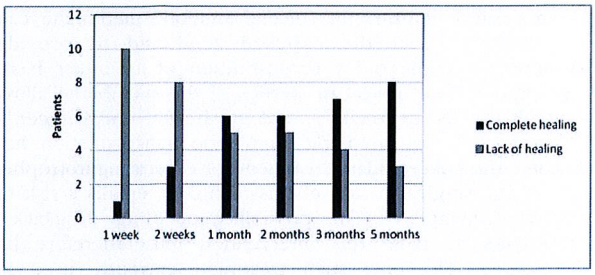

Figure 3. Change in the number of patients displaying complete corneal healing during RGTA treatment.

for all patients went from 1.98 ± 0.51 to 1.79 ± 0.54 after RGTA treatment discontinuation ($P = 0.152$); for the eight patients with complete corneal healing, mean LogMAR visual acuity went from 2.175 ± 0.35 to 1.91 ± 0.57 after RGTA treatment discontinuation ($P = 0.13$) .

None of the patients reported pain or discomfort during instillation of the drops.

Discussion

Corneal healing is a complex process involving cellular interaction and various molecules (proteases, growth factors, and epithelial and stromal cytokines).[9,10] Corneal neurotrophic ulcers, particularly in patients with total corneal anesthesia, are among the most difficult ophthalmological conditions to treat, and may potentially result in blindness. In the absence of healing, they progress toward corneal perforation or total de novo vascularization. Perforation is the final outcome of a process of cellular degradation resulting from chronic inflammation.[11–13]

Many neurotrophic ulcers are of iatrogenic origin (e.g., nonsteroidal anti-inflammatory drops),[14] and thus may improve following cessation of the treatment in question. It should be stressed that we included in our series only patients for whom elimination of toxic treatments for corneal epithelium and artificial tears instillation for 15 days had been unsuccessful.

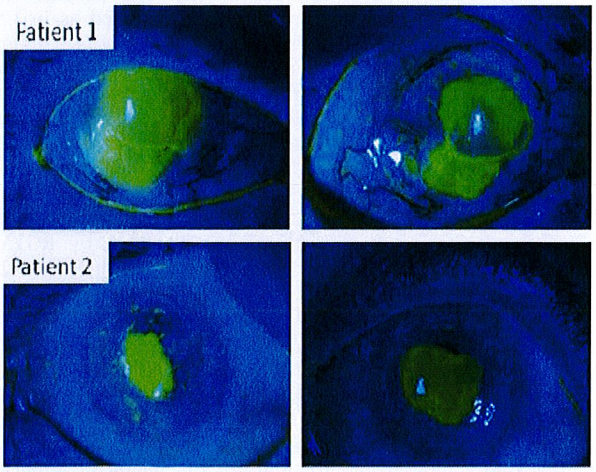

Figure 4. Patients who were nonresponders to RGTA treatment. *Left column:* before treatment; *right column:* at the end of follow-up. Patient 1: corneal neurotrophic ulcer secondary to neovascular glaucoma. Patient 2: corneal neurotrophic ulcer secondary to acanthamoeba keratitis.

In corneal neurotrophic ulcers, amniotic membrane can be used to cover the exposed zone and to provide components required for reconstitution of an intact basal membrane. The physical properties of this membrane allow epithelial cells to recover and to heal corneal ulcer.[15] However, although amniotic membrane transplantation has become the gold standard treatment for corneal neurotrophic ulcers, the surgical nature of this technique entails a risk of central scarring.[16,17] The possibility of using drug-based treatments to avoid this intervention must therefore be considered. The only such treatment currently used in clinical practice and shown to be effective is autologous serum.[18] However, its use may be difficult due to the requirement for weekly blood sampling and for a specific preparation procedure. The other disadvantages of this technique include risk of contamination for patients and care staff, instability of the product, absence of a legal framework for this approach, need for very close collaboration between the blood bank and the ophthalmologist, and its cost.[19–22] Instillation of NGF could also be envisaged. Topical NGF use shows encouraging results in clinical trials[23,24]; however, its use is not straightforward, because it is not currently available due to its animal origin. Heparan-sulfate mimetic may be a possible alternative therapy to autologous serum or amniotic membrane transplantation. Glycosaminoglycans, including heparin sulfates in particular, are involved in intercellular communication and contribute to the regulation of cellular homeostasis. Indeed, heparin sulfates are involved in the anchoring and protection of various growth factors. They also participate in the architecture of the matrix, by linking various structural proteins (e.g., collagen, elastin, and fibronectin).[25] The effects of RGTA on skin ulcers, corneal ulcers, and burns have been investigated. When a tissue is attacked, stressed cells release proteases, which destroy this matrix architecture. Tissue-regenerating agents (RGTA) mimic the action of destroyed heparan-sulfate molecules, thereby recreating a matrix microenvironment in which cells can migrate and multiply. Moreover, these agents break the negative repair-destruction cycle occurring in chronic lesions.[26] RGTA has also been reported to have an antifibrotic effect, through decrease in collagen III synthesis and improvement in collagen reorganization. RGTA also inhibits proteolytic enzymes in vitro.[27] RGTA, (Cacicol20) is supplied as a sterile single-dose solution of alpha 1-6 polycarboxymethyl glucose sulfate described and synthesized as in US Patent Number 6689741, with dextran T40 and 0.9% sodium chloride as excipients. It contains no component of animal or biological origin, and penetrates into the cornea without crossing Descemet's membrane. Moreover, this treatment is necessarily safer in terms of sterility and manufacturing origin than those local treatments developed to date. Dosage was chosen according to previous animal study on skin.[28] The number of heparan-binding sites available in wound tissue is limited, and once all these sites are occupied by RGTA, excess RGTA may compete with heparan-binding growth factors for sites on the matrix-bound RGTA. Thus, heparan-bound growth factors/cytokines stored in the matrix could be removed from the matrix by this excess, hence reducing the amount of growth factor available and healing efficacy. For this reason, daily, or more than daily, addition does not seem to be either efficient or even effective, while the best dosage was once every second or third day. However, no specific studies were undertaken to optimize the timing of applications.

Our results show that the approach of targeting extracellular matrix can be effective in the reepithelialization of neurotrophic ulcers that do not respond to usual treatment. Indeed, we showed the efficacy of this new topical, noninvasive treatment in 8 of the 11 patients tested, with total reepithelialization occurring within a period of 1 to 22 weeks. One patient suffered a recurrence 4 months after total healing, necessitating reintroduction of RGTA treatment; the rate of recurrence was thus low, particularly as anesthesia persisted in all cases. However, continuation of treatment after complete reepithelialization does not appear to be useful, given the properties of the product, whereas use of NGF or autologous serum could be continued for long courses of curative treatment and subsequently for preventive treatment.

We noted three cases of treatment failure in this series. In two of these cases, the ulcers worsened during RGTA treatment. Failure of this treatment in one patient with an ulcer secondary to neovascular glaucoma could be accounted for by presence of calcareous deposits, preventing cell adhesion and migration. Treatment also failed in a patient with acanthamoeba keratitis who had already undergone amniotic membrane transplantations. This ulcer progressed toward perforation, requiring emergency penetrating keratoplasty. The severity of this case may explain the poor response to treatment. One patient, whose ulcer was very deep but healed after a longer period (14 weeks), finally presented a central corneal microperforation. This case was therefore scored as a case of treatment failure despite the gradual healing of the ulcer. This patient had rheumatoid arthritis affecting several joints and we suspect that treatment failure in this case was due to the inflammatory nature of the disease.

One of the limitations of this study was the lack of randomization, but such randomization would be feasible only in the framework of a multicenter trial allowing recruitment of a sufficient number of cases.

Finally, our study suggests that this approach may be useful for the treatment of corneal neurotrophic ulcers. Additional randomized studies are now required to confirm these encouraging results.

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